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BRAIN REGULATION OF REPRODUCTION IN TELEOSTS

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R. E. Peter, V. L. Trudeau and B. D. Sloley (1991) Brain regulation of reproduction in teleosts. *Bull. Inst. Zool., Academia Sinica, Monograph* **16**: 89-118. The neuroendocrine regulation of reproduction in teleosts is a complex and interactive process. Environmental and pheromonal cues are perceived and interpreted by the brain which is involved in both stimulatory and inhibitory regulation of gonadotropin release from the pituitary. Gonadotropic hormones released by the pituitary stimulate gonadal maturation and, in turn, gonadal steroids feedback to both the brain and pituitary to modulate the release of gonadotropin. This paper will describe recent work involving the regulation of gonadotropin-II (GtH) secretion by gonadotropin-releasing hormone (GnRH) peptides and the neuroendocrine control of GnRH release from the brain and pituitary. The influence of dopamine and other neurohormones or neurotransmitters on both GtH and GnRH secretion, and the role of sex steroids in controlling GtH secretion through feedback mechanisms will also be discussed. Use of the information derived from this research has implications for application in the control of fish reproduction in aquaculture.

Key words: Gonadotropin-releasing hormone, Dopamine, Sex steroid feedback, Ovulation, Induced spawning.

The secretion of gonadotropin-II (GtH) in teleosts is under a combined stimulatory and inhibitory neuroendocrine regulation. The primary neuroendocrine factor recognized as a stimulator is gonadotropin-releasing hormone (GnRH) and the primary inhibitor of GnRH-stimulated GtH release is dopamine (Peter *et al.*, 1986). However, a number of other neurohormones, and sex steroids also influence GtH secretion. The first purpose of this review is to examine the regulation of GtH secretion by GnRH and dopamine, as well as the role other neuroendocrine factors may play in the stimulation or inhibition of GtH secretion in teleosts. The second purpose is to discuss the practical application, or the

potential application, of this knowledge of neuroendocrine regulation to fish culture. The third purpose is to project the future directions of both basic and applied research in this area, and the implications this research may have for control of reproduction in aquacultural species.

STIMULATION OF GONADOTROPIN-II SECRETION BY GnRH PEPTIDES

The [Trp^7, Leu^8]-GnRH (salmon GnRH, sGnRH) and [His^5, Trp^7, Leu^8]-GnRH (chicken GnRH-II, cGnRH-II) forms of GnRH are found in a wide range of teleost species; in most species investigated unidentified minor forms and in some cases different major forms are present (Peter *et al.*, 1987a, Sherwood, 1987; Sherwood and Lovejoy, 1989). sGnRH and cGnRH-II are active in stimulating GtH release in goldfish both *in vivo* (Peter *et al.*, 1987b) and *in vitro* (MacKenzie *et al.*, 1984; Chang *et al.*, 1990a). Of particular interest, cGnRH-II is more potent than sGnRH in stimulating GtH release from goldfish dispersed pituitary cells cultured under static conditions (Chang *et al.*, 1990a). The other forms of GnRH present in vertebrates, mammalian GnRH (mGnRH), chicken GnRH-I (cGnRH-I) and lamprey GnRH (lGnRH) also stimulate GtH secretion *in vivo* in goldfish (Peter *et al.*, 1985, 1987b); however, the relative potencies of the peptides have not been quantified. The GtH releasing activity of sGnRH has been demonstrated in several teleost peciess both *in vivo* and *in vitro*, including salmonids (Weil *et al.*, 1986; Van Der Kraak *et al.*, 1987; Weil and Marcuzzi, 1990a, 1990b), African catfish, *Clarias gariepinus* (De Leeuw *et al.*, 1988a, 1988b) and gilthead seabream, *Sparus aurata* (Zohar *et al.*, 1989). Notably, sGnRH and mGnRH are rapidly degraded by enzymes in the pituitary, kidney and liver of gilthead seabream (Goren *et al.*, 1990; Zohar *et al.*, 1989, 1990), which limits the effectiveness of native forms *in vivo*. The substitution of selected D-amino acids in position-6 of GnRH provides resistance to cleavage between positions 5 and 6 (Karten and River, 1986). There is also a cleavage site between Pro^9-Gly^{10}-NH_2 in GnRH, and substitution of ethylamide for amino acid 10 stabilizes the C-terminal of mGnRH in mammals (Karten and Rivier, 1986), and of sGnRH and mGnRH in the gilthead seabream (Zohar *et al.*, 1989, 1990). Structure-activity relations of analogs of mGnRH and sGnRH were

investigated *in vitro* in goldfish, and the Pro^9-N ethylamide alteration was found to increase potency of both mGnRH and sGnRH (Habibi *et al.*, 1989b). Position-6 substitution of D-amino acids also increased potency of mGnRH and sGnRH in the goldfish (Habibi *et al.*, 1989b); however, although substitution of hydrophobic D-amino acids in position-6 generally increased the potency of mGnRH analogs in goldfish similar to the situation in mammals (Karten and Rivier, 1986), the presence of Trp^7 in sGnRH makes it hydrophobic relative to mGnRH and further increases in hydrophobicity do not appear to confer an advantage (Habibi *et al.*, 1989b).

In studies on the structure-activity relations of agonist analogs of mGnRH and sGnRH *in vivo* in goldfish, [D-Arg^6, Pro^{9}NEt]-sGnRH (sGnRH-A) was discovered to be the most active of all those tested (Peter *et al.*, 1985; Peter, 1986). In the gilthead seabream sGnRH-A was found to be highly resistant to enzymatic degradation (Zohar *et al.*, 1990), one of the factors contributing to the high potency of this analog *in vivo*. Another factor important in determining potency of GnRH analogs is affinity for pituitary receptors (Karten and Rivier, 1986). High affinity/low capacity and low affinity/high capacity binding sites for GnRH peptides were described in ligand binding and displacement studies using goldfish pituitary membrane preparations (Habibi *et al.*, 1987, 1989a, b). The high affinity binding sites are believed to be involved in activation of the hormone secretion responses, as the potencies of the peptides for stimulating GtH release *in vitro* and binding affinities are in corresponding nanomolar ranges, and some peptides active in releasing GtH do not bind to the low affinity binding sites (Habibi *et al.*, 1989b; Peter *et al.*, 1990). Also, for analogs of sGnRH, a strong correlation was found between binding to the high affinity sites and potency of GtH releasing activity from goldfish pituitary fragments in perifusion culture, with sGnRH-A having both the highest affinity and potency (Habibi *et al.*, 1989b). The high affinity/low capacity and low affinity/high capacity GnRH binding sites were shown to be of two different molecular sizes in photoaffinity labelling experiments, demonstrating their distinctive nature (Habibi *et al.*, 1990). In African catfish pituitary, high affinity/low capacity GnRH binding sites have been described (De Leeuw *et al.*, 1988a); although

sGnRH-A had the highest affinity to GnRH binding sites, sGnRH-A and [D-Ser(t-Bu)^6, Pro^{9}NEt]-mGnRH (Buserelin) were equipotent in stimulating GtH release from perifused catfish pituitary fragments (De Leeuw *et al.*, 1988b). Crim *et al.* (1988a, 1988b), investigated the activities of a number of mGnRH and sGnRH analogs for binding to high affinity/low capacity sites in the winter flounder pituitary, GtH release *in vivo* in sexually mature male landlocked Atlantic salmon, and GtH release *in vivo* and *in vitro* in testosterone pretreated immature rainbow trout; sGnRH-A was consistently amongst the most potent analogs tested in each of these assay systems. Given that cGnRH-II is more potent than sGnRH in stimulating GtH release from goldfish pituitary cells cultured under static conditions (Chang *et al.*, 1990a), it would be of interest to test a series of position 6 D-amino acid analogs of cGnRH-II for GtH releasing activity in different teleosts, to determine if more potent analogs than sGnRH-A can be developed.

sGnRH-A and [D-Ala^6, Pro^{9}NEt]-mGnRH (LHRH-A), given alone or in combination with the dopamine antagonist pimozide, have been compared for potency and effectiveness in stimulating serum GtH levels and ovulation in goldfish (Peter *et al.*, 1985, 1987c). sGnRH-A was found to be about ten-fold more potent than LHRH-A under these conditions in the goldfish. In the common carp and the Chinese loach, sGnRH-A was also about ten-fold more potent than LHRH-A in stimulating serum GtH levels and ovulation (Lin *et al.*, 1988, 1991b).

The seasonal changes in responsiveness of goldfish to LHRH-A have been tested (Sokolowska *et al.*, 1985b; Habibi *et al.*, 1989a; Trudeau *et al.*, 1991b). The greatest increases in serum GtH to LHRH-A given either alone or in combination with the dopamine antagonist primozide were found in goldfish that had completed gonadal recrudescence (=prespawning), and fish that were sexually regressed were the least responsive. This seasonal peak in responsiveness coincides with the seasonal peak in capacity of both the high and low affinity GnRH receptors in the goldfish pituitary (Habibi *et al.*, 1989a). This suggests that receptor capacity may in part determine responsiveness to GnRH analogs.

Down-regulation of GnRH high affinity binding sites occurs *in vitro* following a 120 minute exposure of the goldfish pituitary to sGnRH or

cGnRH-II, but not following exposure to the antagonist [D-pGlu[1], D-Phe[2], D-Trp[3,6]]-mGnRH (Habibi, 1991). cGnRH-II has a higher affinity for the high affinity binding sites than sGnRH, and it caused a greater down-regulation of the high affinity binding sites than sGnRH. This receptor down-regulation was associated with a subsequent desensitization of the GtH response to a second exposure to sGnRH or cGnRH-II. This is the first demonstration of down-regulation of GnRH receptors in a teleost. How important receptor down-regulation and desensitization of the hormone release response might be when fish are exposed to GnRH analogs over extended periods is not known. Notably, when goldfish (Peter *et al.*, 1985, 1987b), common carp (Lin *et al.*, 1987a, 1988) and Chinese loach (Lin *et al.*, 1987b, 1988, 1991b) are injected with sGnRH-A, either alone or in combination with a dopamine antagonist, serum GtH levels remain elevated for periods well past 24 hours in each species. Whether, in such circumstances, there is GnRH receptor down-regulation is not known; however, it is clear that GtH release does not become completely desensitized. In goldfish, at 24 hours following the last of three injections 48 hours apart of sGnRH-A, serum GtH levels were greatly increased and the capacity of the high affinity GnRH binding sites was also increased (Omeljaniuk *et al.*, 1989a). This suggests that over the longer term, GnRH receptor capacity may increase, rather than decrease, in response to prolonged exposure to GnRH peptides. The influences of GnRH peptides on GnRH receptor capacity clearly needs further study in teleosts.

In many teleosts dopamine has a direct effect on gonadotrophs to inhibit GnRH-stimulated GtH release (Peter *et al.*, 1986; Chang *et al.*, 1990b; reviewed below). This inhibitory effect on stimulated GtH release is effective within seconds of exposure of pituitary cells to dopamine or the dopamine agonist apomorphine (unpublished results). Dopamine also influences responsiveness to GnRH peptides by effects on GnRH receptor capacity. Omeljaniuk *et al.* (1989a) reported that injection of goldfish with the dopamine type-2 (D-2) receptor antagonist domperidone caused an increase 24 hours later in GnRH receptor capacity in the goldfish pituitary. De Leeuw *et* al. (1989) demonstrated the dose- and time-dependency of this up-regulation effect of domperidone on GnRH receptors in the goldfish pituitary.

Furthermore, the GnRH receptor capacity of the goldfish pituitary could also be influenced by treatments *in vitro* with apomorphine and domperidone, indicating that these are due to direct effects on the gonadotrophs. The apparent effects of increased dopamine input to decrease GnRH receptor capacity, and decreased dopamine input to increase GnRH receptor capacity provides a means for longer term influences of dopamine on GnRH responsiveness.

A factor that will influence the effectiveness of GnRH peptides *in vivo* in teleosts is the metabolic clearance rate of the peptides. While resistance to enzymatic degradation is certainly one of the factors influencing the metabolic clearance rate, the presence and affinity of GnRH peptides for a serum binding protein would be another important factor. A serum GnRH binding protein was first described in the goldfish (Huang and Peter, 1988). More recently, the goldfish serum GnRH binding protein has been partially purified, affinity labelled with ^{125}I-[Dys^{6}, Pro^{9}NEt]-sGnRH and the molecular weight estimated at 40,000 kilodaltons (Huang *et al.*, 1991a). The GnRH binding protein specifically binds sGnRH, cGnRH-II and analogs of sGnRH, but not mGnRH and LHRH-A (Huang *et al.*, 1991a). The binding affinities of sGnRH-A>sGnRH, and the lack of any specific binding of mGnRH (Huang *et al.*, 1991a), correlates with the metabolic clearance rates of sGnRH-A>sGnRH>mGnRH (Huang *et al.*, 1991b). The affinities of sGnRH and sGnRH-A for the serum binding protein are about three orders of magnitude less than for the pituitary high affinity binding sites. The titer of the GnRH binding protein in goldfish serum is in excess relative to the concentrations of circulating sGnRH and cGnRH-II in goldfish (Peter *et al.*, 1990; Huang *et al.*, 1991c). On this basis it is presumed that the physiological function of the binding protein is to slow the clearance of GnRH peptides in circulation. In the case of injected GnRH analogs, the serum GnRH binding protein would also serve to extend the treatment of analogs that bind to it, by slowing the metabolic clearance rate of the analog. GnRH peptides bound to the binding protein will be readily available to bind to the receptors, because of the differences in binding affinities of the pituitary GnRH receptors and the serum binding protein. The importance of this to regulation of GtH

release from the pituitary is not clear as GnRH peptides are already delivered to the pituitary by means of innervation by neurosecretory fibers. However, GnRH peptides have inhibitory effects on progestogen-induced oocyte meiosis (Habibi *et al.*, 1988) and GtH-induced testosterone production (Habibi *et al.*, 1989c) of goldfish oocytes, suggesting that GnRH peptides bound to the serum binding protein may have the gonads as the primary target.

REGULATION OF GONADOTROPIN-RELEASING HORMONE RELEASE

An inverse relationship between brain GnRH and plasma GtH levels has been reported in wild-caught male and female roach (*Rutilus rutilus*) during the spawning period (Breton *et al.*, 1988a, 1988b); however, the precise timing of ovulation and spawning could not be determined in this field study. Female goldfish have a depletion of the GnRH concentrations in the olfactory bulbs, telencephalon plus preoptic region, hypothalamus and pituitary coincident with the onset of the ovulatory surge in serum GtH levels, with repletion back to starting concentrations occurring by the end of the ovulatory surge of GtH some 12 to 16 hrs later (Yu *et al.*, 1987, 1991a). Male goldfish accompanying ovulating females also have a surge in circulating blood levels of GtH lasting about 12 hrs (Kobayashi *et al.*, 1986; Yu *et al.*, 1991a) in response to pheromone signals from the female, specifically the oocyte maturation steroid 17α,20β-dihydroxy-4-pregnen-3-one (Dulka *et al.*, 1987) and prostaglandin-$F_{2\alpha}$ (Kyle *et al.*, 1985; Yu and Peter, 1990a). Such male goldfish accompanying ovulating females also have a rapid depletion and repletion of brain GnRH concentrations (Yu *et al.*, 1991a). Notably, these pheromone signals can be given independent of each other to induce GtH responses from the males; addition of 17α, 20β-dihydroxy-4-pregnen-3-one to the water induces a surge release of GtH within 15 minutes in males (Dulka *et al.*, 1987). The changes in brain GnRH concentrations that may occur during the large and rapid surge in serum GtH levels following exposure to 17α, 20β-dihydroxy-4-pregnen-3-one have not been investigated. Exposure of males to females injected with prostaglandin-$F_{2\alpha}$ results in spawning behavior and release of some unknown pheromone signal, an increase in concentrations of GnRH in the

olfactory bulbs, telencephalon and hypothalamus lasting about 2 hours, and a small but significant increase in blood levels of GtH lasting 4 hours in the males (Yu and Peter, 1990a). A final point regarding the changes in brain and pituitary GnRH concentrations during spawning or pheromone exposure in the goldfish is that the changes tend to occur in the entire forebrain plus pituitary, irrespective of whether the change is an increase or a decrease. This indicates that although the GnRH perikarya and fibers are widely distributed in the forebrain of the goldfish (Kah *et al.*, 1986a), the network of GnRH perikarya and fibers can be regarded as an integrated neuronal system.

Evidence from *in vivo* studies on goldfish indicates that dopamine has an inhibitory input to the GnRH neuronal system to regulate brain concentrations of GnRH as well an inhibitory input on release of GnRH (Yu and Peter, 1990b). Treatment of intact male goldfish with the dopamine antagonist pimozide causes a dose- and time-dependent increase in GnRH concentrations in the olfactory bulbs, telencephalon and pituitary that can be blocked by co-treatment with the dopamine agonist apomorphine. The short-term increase in concentrations of brain GnRH and serum GtH in males spawning with females injected with prostaglandin-$F_{2\alpha}$ can also be blocked by apomorphine. In males treated with pimozide and spawning with females injected with prostaglandin-$F_{2\alpha}$ there is a marked depletion of the pimozide-elevated brain GnRH concentrations and a large surge in serum GtH levels. The hypothesis concerning the inhibitory role of dopamine on GnRH release has been confirmed by *in vitro* studies (Yu and Peter, 1991; Yu *et al.*, 1991b). Dopamine inhibits GnRH release from preoptic-anterior hypothalamic brain slices and pituitary fragments from female (Yu *et al.*, 1991b) and male (Yu and Peter, 1991) goldfish. The pituitary-level inhibition of GnRH release is by dopamine type-2 receptors (D-2), and is thought to be by axo-axonal interactions of dopamine fibers on GnRH fibers (Yu and Peter, 1991). The dopamine inhibition of GnRH release from preoptic-anterior hypothalmic slices is by type-1 receptors; the specific site of this inhibitory input to the GnRH neuronal system is not known.

Serotonin has dose dependent stimulatory effects on GnRH release from goldfish preoptic-anterior hypothalmic slices and pituitary fragments, suggesting serotonergic

axo-axonal contact with GnRH fibers in the pituitary and serotonergic input to the brain GnRH neuronal system (Yu *et al.*, 1991b). In addition, norepinephrine has a stimulatory effect on GnRH release from preoptic-anterior hypothalamic slices, but no effect on GnRH release from pituitary fragments (Yu and Peter, 1991; Yu *et al.*, 1991b). This noradrenergic input to the brain GnRH neuronal system appears to be by α_1-type receptors (Yu and Peter, 1991).

On the basis of this information we can begin to develop a model for the regulation of the GnRH neuronal system and GnRH release in the goldfish. It is particularly notable that dopamine has additional inhibitory functions in the regulation of GtH secretion, in this case the regulation of GnRH release. However, goldfish have at least two forms of GnRH in the brain and pituitary, sGnRH and cGnRH-II (Yu *et al.*, 1988), and due to limitations of the radioimmunoassay system used in the above studies we were only able to quantify total GnRH concentrations (Yu *et al.*, 1987, 1991a). Given that the two forms of GnRH in goldfish have a differential distribution in the brain (Yu *et al.*, 1988), and that the two forms have some differences in activity in stimulating the release of GtH as well as growth hormone (Marchant *et al.*, 1989; Chang *et al.*, 1990a), it clearly would be of importance to directly study the regulation of release of each of the two forms of GnRH in the goldfish. In rainbow trout sGnRH and cGnRH-II also have a differential distribution in the brain, with sGnRH being predominant in all brain regions except the cerebellum, and sGnRH alone being found in the pituitary (Okuzawa *et al.*, 1990). Given the importance of monoamine neurotransmitters in the direct regulation of GtH secretion in teleosts (see below), as well as in the regulation of GnRH in goldfish, it clearly would also be of importance to study the dynamics of the various monoamine neurotransmitters in order to more fully understand the regulation of GnRH release.

DOPAMINE INHIBITION OF GONADOTROPIN SECRETION

The direct inhibition by dopamine of GnRH-stimulated GtH release and spontaneous release of GtH in goldfish was first demonstrated by Chang *et al.* (1984a) in a study using enzymatically dispersed pituitary cells and pituitary fragments in

perifusion culture. Injection of dopamine and the dopamine agonist apomorphine into goldfish with a transplanted pars distalis of the pituitary gland significantly reduced serum GtH levels (Chang *et al.*, 1984b), again indicating that dopamine has a direct inhibitory effect on spontaneous secretion of GtH. The specificity of the inhibitory effects on GtH release to dopamine, versus other catecholamines, was confirmed in studies using a number of synthesis inhibitors and receptors antagonists (Chang *et al.*, 1984c, Peter *et al.*, 1986). Chang *et al.* (1984c) reported that the effects of injected LHRH-A on serum GtH levels in goldfish can be suppressed by injection of apomorphine or potentiated by injection of dopamine receptor antagonist drugs; although LHRH-A alone was not effective in inducing ovulation in the goldfish, the combination of dopamine antagonist and LHRH-A was highly effective. This latter finding led to the development of a new technique for induced ovulation of cultured fish.

The specificity of the dopamine inhibition of GtH release in goldfish was investigated in a series of *in vivo* and *in vitro* studies. Specific dopamine D-2 antagonist drugs were effective in potentiating the GtH releasing actions of LHRH-A or other GnRH peptides in goldfish, with pimozide and domperidone being the most potent (Peter *et al.*, 1986; Omeljaniuk *et al.*, 1987). The dose dependency of the dopamine inhibition of sGnRH-stimulated GtH release from perifused goldfish pituitary fragments, and the blockage of these dopamine effects by domperidone, were demonstrated by Omeljaniuk *et al.* (1989b). The GtH release stimulated by sGnRH and cGnRH-II from dispersed goldfish pituitary cells in static and perifusion culture can be blocked by D-2 receptor agonists and this inhibition reversed by D-2 antagonists; however, dopamine type-1 receptor (D-1) agonists and antagonists have no effects on GtH release (Chang *et al.*, 1990b). Finally, the specificity of the D-2 receptors have been demonstrated in receptor binding studies on the goldfish pituitary (Omeljaniuk and Peter, 1989).

On the basis of brain lesioning studies on goldfish, Peter and Paulencu (1980) first suggested the presence of a GtH release-inhibitory factor originating in the preoptic region and coursing to the pituitary along tracts in the ventral anterior hypothalamus. Kah *et al.* (1984) demonstrated by immunocytochemistry the presence of a preoptic

dopaminergic nucleus and a preoptic-hypophysial dopaminergic pathway in the goldfish. The gonadotrophs in goldfish receive direct innervation by dopaminergic fibers (Kah *et al.*, 1986b), and this innervation is abolished by lesioning the anterior ventral preoptic region of the goldfish (Kah *et al.*, 1987b). These studies provide an anatomical substrate for the GtH release inhibitory activities of dopamine in the goldfish.

The presence of dopamine inhibition of GtH release has been extended to a number of other teleost species. In African catfish, apomorphine was demonstrated to inhibit GtH release stimulated by a GnRH analog from perifused pituitary fragments and cell suspensions (De Leeuw *et el.*, 1986). In *in vivo* studies on African catfish, a number of dopamine and serotonin antagonists were tested for ability to potentiate the GtH response to LHRH-A, and only the dopamine antagonists, particularly Org 5222 and Org 30067, were effective (Goos *et al.*, 1987). In other studies it was shown that the D-2 agonist bromocryptine had an inhibitory effect on GnRH analog-stimulated GtH release, whereas the D-2 antagonoists sulpiride and domperidone potentiated the response; D-1 agonist and antagonists drugs did not influence the responsiveness (Van Asselt *et al.*, 1988). The pituitary D-2 receptors in the catfish pituitary have been characterized and specificity defined in receptor binding studies (Van Asselt *et al.*, 1990). In tilapia the *in vivo* GtH response to LHRH-A is potentiated by treatment with pimozide (Gissis *et al.*, 1988), and dopamine has been shown to inhibit GtH secretion from perifused pituitary fragments stimulated by LHRH-A or the calcium ionophore A23187 (Yaron and Levani-Sivan, 1990).

In the Chinese loach, a number of catecholamine synthesis blockers and receptor antagonist drugs, alone and in combination with LHRH-A, have been used to demonstrate the presence of dopamine inhibition on GtH release (Lin *et al.*, 1985, 1986, 1988). The specificity of the dopamine inhibition to D-2 receptors was shown by the reversal by domperidone of dopamine inhibition of sGnRH-A stimulated GtH release (Lin *et al.*, 1989). From comparative studies on common carp and Chinese loach, Lin *et al.* (1988) concluded that the dopamine inhibitory tone on GtH release was less prominent in the loach than in common carp; there was less potentiation of sGnRH-A on

GtH release by the antagonists pimozide and domperidone, and greater responsiveness to sGnRH-A alone in the loach than in the common carp.

Notably, in the Atlantic croaker (*Micropogonias undulatus*) there is no evidence indicating a dopamine inhibitory regulation on GtH secretion (Copeland and Thomas, 1989). In a series of *in vivo* experiments the effects of injection of a number of dopamine agonist and antagonist drugs on the GtH response to LHRH-A were tested and on evidence of inhibition or potentiation, respectively, was found.

The dopamine antagonist pimozide potentiates the effects of LHRH-A or sGnRH-A on GtH release in goldfish, and the combined treatment is highly effective in inducing ovulation (Chang *et al.*, 1984c, Sokolowska *et al.*, 1985a, 1985b; Peter *et al.*, 1987c). Similarly, in common carp (Billard *et al.*, 1983; Lin *et al.*, 1986, 1987a, 1988), Chinese loach (Lin *et al.*, 1987b, 1988, 1991b) and African catfish (De Leeuw *et al.*, 1985a, 1985b) the dopamine antagonists pimozide or domperidone potentiate the effects of LHRH-A or sGnRH-A on GtH levels in the blood, and the combined treatment is highly effective in inducing ovulation. The GtH-release inhibitory action of dopamine has also been demonstrated in European eel (Dufour *et al.*, 1984, 1988), rainbow and brown trout (Billard *et al.*, 1984), Chinese bream (Lin *et al.*, 1986), coho salmon (Van Der Kraak *et al.*, 1986), and tilapia (Gissis *et al.*, 1988). The combined treatment of dopamine antagonist and GnRH agonist for induced ovulation has been termed the Linpe method (Peter *et al.*, 1988a); the Linpe method has proven highly successful for induced ovulation of a number of aquaculture species, including common carp, grass carp, silver carp, bighead carp, black carp, mud carp, Thailand mud carp, mandarinfish, Chinese silvery chub, Chinese loach, Chinese catfish, African catfish and South American pacu (Lin *et al.*, 1986; Peter *et al.*, 1988a, 1988b; Lin and Peter, 1990). The Linpe method is now commercialized as domperidone and LHRH-A in P.R. China, and commercialized as Ovaprim (domperidone and sGnRH-A) by Syndel Laboratories, Canada. This technique for induced ovulation of cultured fish has a number of advantages over traditional methods, including reduced cost of synthetic drugs, long stability of the drugs in storage, high predictability of the time from injection to ovulation, high fertility of eggs, high

survival of fry, decreased stress on broodstock due to the necessity for only a single injection, and lack of any apparent side effects on the reproductive or immune systems (Peter *et al.*, 1988a, 1988b; Lin and Peter, 1990).

FEEDBACK EFFECTS OF SEX STEROIDS

Gonadectomy causes increased blood GtH levels in rainbow trout (Bommelaer *et al.*, 1981), African catfish (Habibi *et al.*, 1989a) and goldfish (Kobayashi and Stacey, 1990) that are suppressible by replacement therapy with estradiol and/or testosterone, providing the classical demonstration of negative feedback of sex steroids in teleosts. Implantation of antiestrogens (ICI 46474 and clomiphene) into the brain and pituitary of goldfish caused an increase in serum GtH levels (Billard and Peter, 1977), providing evidence for steroid negative feedback directly on the brain-pituitary axis; however, implantation into the pituitary may have damaged the pituitary stalk and disrupted inhibitory dopaminergic inputs to the pituitary (Peter *et al.*, 1986) and caused GtH release.

The mechanisms underlying sex steroid negative feedback have not received a great deal of attention; however, recent studies suggest that both the neurotransmitter γ-aminobutryic acid (GABA) and dopamine may be involved. There is a prominent innervation of the goldfish pituitary by GABA (Kah *et al.*, 1987a), suggesting a possible neuroendocrine function. Injection of GABA in goldfish causes an increase in serum GtH levels, which may be due a stimulatory effect of GABA on GnRH release from GnRH nerve terminals in the pituitary (Kah *et al.*, 1990). Treatment of sexually regressed and recrudescent goldfish with estradiol, but not testosterone, reduced brain GABA concentrations (unpublished results) and abolished the stimulatory effects of GABA on GnRH release from goldfish pituitary fragments *in vitro* (Kah *et al.*, 1990). The inhibitory effects of estradiol on GABA actions and levels may be a part of the mechanism of estrogen negative feedback on the brain-pituitary axis in the goldfish. The negative feedback effects of sex steroids could also be mediated through the inhibitory dopaminergic effects on GnRH release and on GtH release; treatment of goldfish with estradiol or testosterone, caused an increase in the dopamine turnover rates in the

telencephalon-preoptic brain region and the pituitary of female goldfish undergoing ovarian recrudescence (unpublished results). This suggests that sex steroids may increase the dopamine inhibitory tone on GnRH and and GtH release.

In sexually immature teleosts, sex steroids appear to have a predominantly positive feedback effect. Increased pituitary content of GtH occurs following treatment of juvenile male and female rainbow trout with testosterone (Crim and Evans, 1979). This positive feedback effect of androgens on pituitary GtH content is dependent on aromatization to estrogens (Crim *et al.*, 1981), and it is also reflected by an enhanced responsiveness of the pituitary to GnRH *in vitro* (Crim and Evans, 1980). Whether this positive feedback effect of sex steroids is a part of the mechanisms underlying puberty is not known; however, following prolonged treatment of immature trout with testosterone, GtH could be detected in the plasma and gonadal development initiated (Crim and Evans, 1983), indicating that the brain-pituitary-gonad axis had become functional. In the European silver eel, multiple injections of estradiol were also found to cause an increase in pituitary GtH content (Dufour *el al.*, 1983) and brain GnRH levels (Dufour *et al.*, 1985); however, only when the estrogen treatment was combined with a dopamine antagonist drug plus LHRH-A were plasma GtH level and ovarian development stimulated (Dufour *et al.*, 1988). In Japanese silver eels injections of estradiol and testosterone both stimulated an increase in pituitary GtH content and serum GtH levels (Lin *et al.*, 1990); combining the estrogen treatment with LHRH-A, or the dopamine antagonist domperidone, or LHRH-A plus domperidone, further stimulated pituitary and plasma GtH levels. Interestingly, treatment with testosterone alone has proven to be the most effective in terms of stimulating gonadal development in the Japanese silver eels (Lin *et al.*, 1989, 1991a).

Kobayashi *et al.* (1989) reported that ovulatory surge-like release of GtH could be induced in ovariectomized goldfish implanted with testosterone or estradiol and exposed to environmental conditions known to induce ovulation in normal prespawning goldfish. This illustrates that positive feedback also is a component of the actions of sex steroids on the brain-pituitary axis in post-pubertal fish. Implantation of testosterone in

both male and female goldfish, at a dosage sufficient to raise serum levels to those found in ovulatory and spawning goldfish, caused an increase in responsivenss to LHRH-A, as measured by the serum GtH response, at all sexual stages throughout the year (Trudeau *et al.*, 1991b). It has also been demonstrated that testosterone treatment increases the responsiveness to injected LHRH-A in sexually recrudescent and sexually mature female common carp and Chinese loach (Trudeau *et al.*, 1991a). This positive feedback effect of testosterone on the brain pituitary axis of goldfish is dependent on aromatization of androgens to estrogens (Trudeau *et al.*, 1991b). However, implantation of a similar dosage of estradiol increased responsiveness in only sexually regressed and post-spawning females, and only at a four-fold higher dosage was estradiol effective in females in late ovarian recrudescence. Thus, positive feedback effects of sex steroids are a natural part of sex steroid action, and both positive and negative feedback effects of sex steroids must coexist in some balance. We have found that the pituitary taken from goldfish implanted with sex steroids has increased responsiveness to sGnRH or cGnRH-II *in vitro* (unpublished results), suggesting that a primary site for the positive feedback action of sex steroids is at the pituitary level. This pituitary level of action of sex steroids is presumably due to an increase in GnRH receptor capacity in the gonadotrophs, although this remains to be investigated.

It has been suggested that the feedback effects of estrogens on GtH secretion are mediated by their conversion, within the catfish brain and/or pituitary to catecholestrogens (CE). DeLeeuw and co-workers (1985c, 1987) have suggested that CE compete with DA for catechol-O-methyl-transferase (COMT) and cause decreased DA degradation and hence increase inhibitory effects on GtH secretion. Indeed, COMT is present in catfish brain and pituitary and, CE and DA compete for COMT *in vitro* (Timmers and Lambert, 1989). However, the levels of CE necessary to compete with DA are far in excess of the CE concentrations expected in vertebrate neural tissues (MacLusky *et al.*, 1981). We have examined the effects of *in vivo* CE treatment on brain and pituitary DA in male goldfish. Injection of 1 μg/g 4-hydroxy-catechol-estradiol (4-OHE_2) at hourly intervals for 5 hrs did not affect brain and pituitary DA levels (unpublished data) nor

did it affect basal or LHRH-A stimulated GtH secretion (Trudeau *et al.*, 1991b). Endogenous CE levels, as determined by HPLC with electrochemical detection, were undetectable in goldfish brain (<100ng/g) and pituitary (<1*p*g/μg protein). Injection with 2-OHE_2 and 4-OHE_2 elevated brain and pituitary CE to measurable levels indicating the effectiveness of *i.p.* injections. We also examined the effects of long term treatments with CE in sexually mature female goldfish. Injection of 1 μg/g 2-OHE_2 or 4-OHE_2 every 2 days for 6 days did not affect basal GtH secretion and generally did not affect LHRH-A induced GtH secretion (unpublished results). At 24h following LHRH-A, however, GtH secretion was higher in fish receiving 4-OHE_2, suggesting that this drug prolongs LHRH-A action. We interpret this slight potentiating effect of 4-OHE_2 as a pharmacological response since the levels of CE used were extremely high. Together, our *in vivo* data in goldfish demonstrate that CE does not inhibit DA degradation nor GtH secretion; as has been suggested for the African catfish (DeLeeuw *et al.*, 1985c, 1987; Timmers and Lambert, 1989).

ROLE OF OTHER NEUROHORMONES IN REGULATION OF GONADOTROPIN SECRETION

Norepinephrine treatment of goldfish, by intraperitoneal or brain intraventricular injection, induces an increase in serum GtH levels in goldfish (Chang and Peter, 1984). This may, in part, be due to the stimulatory effects of norepinephrine on GnRH release (Yu and Peter, 1991; Yu *et al.*, 1991b; reviewed above). Norepinephrine also has a direct effect on GtH release; Chang *et al.* (1991) found that norepinephrine stimulates GtH release from dispersed goldfish pituitary cells in static culture. This effect of norepinephrine could be mimicked by the α-agonist phenylephrine and the α_1-agonist 6-fluoronorepinephrine, but not by other receptor agonists; the effects of norepinephrine could be blocked by the α_1-antagonists prazosine and benoxathian, but not by other receptor antagonists. This demonstrates that the stimulatory effects of norepinephrine on gonadotrophs are *via* α_1-receptors. There is seasonal change in sensitivity of gonadotrophs to norepinephrine, with the GtH response being most sensitive in sexually regressed or regressing fish,

and the least sensitive in sexually recrudescing and prespawning fish. However, the physiological significance of NE direct action on gonadotrophs is unknown since NE is not found in the goldfish pituitary (Sloley *et al.*, 1991). It may be that the α_1-receptors on gonadotrophs respond to changes in NE in the circulation.

Serotonin injection in goldfish causes an increase in serum GtH levels, with the greatest responsivness being in prespawning males and females (Somoza *et al.*, 1988). The stimulatory effect of serotonin on GtH levels was blocked by pretreatment with ketanserin, a specific 5-HT_2 receptor antagonist. *In vitro* serotonin also stimulates GtH release from perifused goldfish pituitary fragments, and the stimulatory effects can be blocked by ketanserin and cyproheptadine (Somoza and Peter, 1991). Whether serotonin has a direct effect on gonadotrophs is not known; however, serotonin does stimulate GnRH release from goldfish pituitary fragments (Yu *et al.*, 1991b), indicating that this may be a part of the mechanism for serotonin stimulation of GtH release.

As indicated above, there is prominent innervation of the goldfish pars distalis of the pituitary by immunopositive GABA fibers (Kah *et al.*, 1987a). Injection of GABA causes an increase in serum GtH levels in goldfish that are in early gonadal recrudescence, but not in fish that are prespawning or sexually regressing (Kah *et al.*, 1990). GABA does not stimulate GTH release from dispersed pituitary cells in static or perifusion culture; however, it does have a stimulatory effect on GnRH release from goldfish pituitary fragments, which explains its stimulatory effects on GtH release (Kah *et al.*, 1990).

Neuropeptide Y (NPY) immunopositive fibers have been found in close association with gonadotrophs in the golfish, suggesting a neuroendocrine function (Kah *et al.* 1989). Porcine NPY (Kah *et al.*, 1989) and human NPY (Peng *et al.*, 1990) stimulate GtH release from perifused fragments of the goldfish pituitary. Pituitary fragments from sexually recrudescing goldfish have a greater magnitude response to NPY than fragments from sexually regressed fish, indicating a seasonal variation in responsiveness (Peng *et al.*, 1990). In rainbow trout on the other hand, NPY has a stimulatory effect on GtH release from perifused pituitaries from ovulated fish, and an inhibitory effect in pituitaries from vitellogenic (=recrudescing) females

(Breton *et al.*, 1989). The stimulatory action of NPY on GtH secretion *in vitro* in goldfish is highly sensitive to apparent receptor down-regulation on both a time and dose-dependent basis (Peng *et al.*, 1990). Preliminary results indicate that NPY directly stimualtes GtH release from dispersed, cultured goldfish pituitary cells (Peng *et al.*, 1990); however, a stimulatory effect on GnRH release is also possible. The amino acid sequence of goldfish NPY has been determined (Larhammar *et al.*, 1990), and it will be of interest to confirm the effects of native goldfish NPY on GtH release in the goldfish.

The endogenous opioid peptides also influence GtH secretion. Treatment of male or female goldfish, in late stages of gonadal recrudescence, with the opioid receptor antagonist naloxone caused a decrease in serum GtH levels and decreased the serum GtH responses to LHRH-A (Rosenblum and Peter, 1989). However, in goldfish in the early stages of gonadal recrudescence naloxone was stimulatory on serum GtH levels when given alone as well as on the responses to LHRH-A; naloxone also potentiated the responses to the dopamine antagonist domperidone in fish at this stage. The mechanisms underlying the interactions of endogenous opioids with gonadotrophs remain to be investigated.

AQUACULTURE PERSPECTIVES

Techniques for induced spawning of many cultured fish, particularly the favored freshwater species, have been improved with the development of the Linpe technique. This technique for spawning induction is receiving growing acceptance in countries around the world. Even species such as the salmonids, which have an apparent low dopamine inhibitory tone on GtH release, can be induced to ovulate and spawn with low dosages of Ovaprim (M. Little, Syndel Laboratories, personal communication). However, whether the Linpe technique is appropriate for marine species that have extended spawning seasons with daily or weekly spawning activity, has not been investigated. Implantation of a GnRH analog in pellets made of biodegradable polymers provides a slow release of the analog, constant elevated blood GtH levels and induces daily spawning for long periods in the seabream, *Sparus aurata* (Zohar, 1989). Injection of LHRH-A or sGnRH-A induced spawning in the sea bass, *Lates calcarifer*, proportional

to the number of daily injections; however, implantation of analog in a cholesterol-cellulose pellet to give extended release induced daily spawning lasting a number of days (Almendras *et al.*, 1988). Additional research will be needed to refine spawning induction techniques for species that have spawning periods extended over a number of days or weeks.

A number of important aquaculture species do not undergo sexual development in the holding ponds. On the basis of recent information concerning sex steroid feedback, it may now be possible to understand how to induce gonadal development in some broodstock by pellet implantation of androgens. Milkfish, *Chanos chanos*, have been successfuly induced to develop to a spawning condition by implantation of pellets containing 17α-methyltestosterone plus LHRH-A (Tamaru *et al.*, 1988). Grey mullet, *Mugil cephalus*, have been induced to undergo gonadal development to a spawning condition by chronic treatment with 17α-methyltestosterone alone (Lee and Tamaru, 1988), and recent studies on the Japanese eel indicate that implantation of pellets containing 17α-methyltestosterone are effective in inducing gonadal development in that species as well (Lin *et al.*, 1991a). These appear to be promising leads in the induction of gonadal development and additional research in this area would be fruitful. However, investigations should emphasize the use of testosterone rather than the anabolic steroid 17α-methyltestosterone; testosterone will be naturally metabolized, whereas 17α-methyltestosterone may accumulate in the tissues. The importance of this is further illustrated by the report that pellet implants of testosterone plus LHRH-A induced ovarian development in striped mullet, *Mugil cephalus*, and that these females could be induced to spawn, whereas there was no ovarian development in females implanted with pellets of similar dosages of 17α-methyltestosterone plus LHRH-A (Tamaru *et al.*, 1989). Moreover, the use of anabolic steroids for stimulation of growth of food animals has been prohibited in many countries.

A problem on which there has been no apparent progress is how to slow gonadal development and delay the spawning period of such prolific species as tilapia. Whether this can be accomplished by treatment with GnRH antagonist analogs requires investigation.

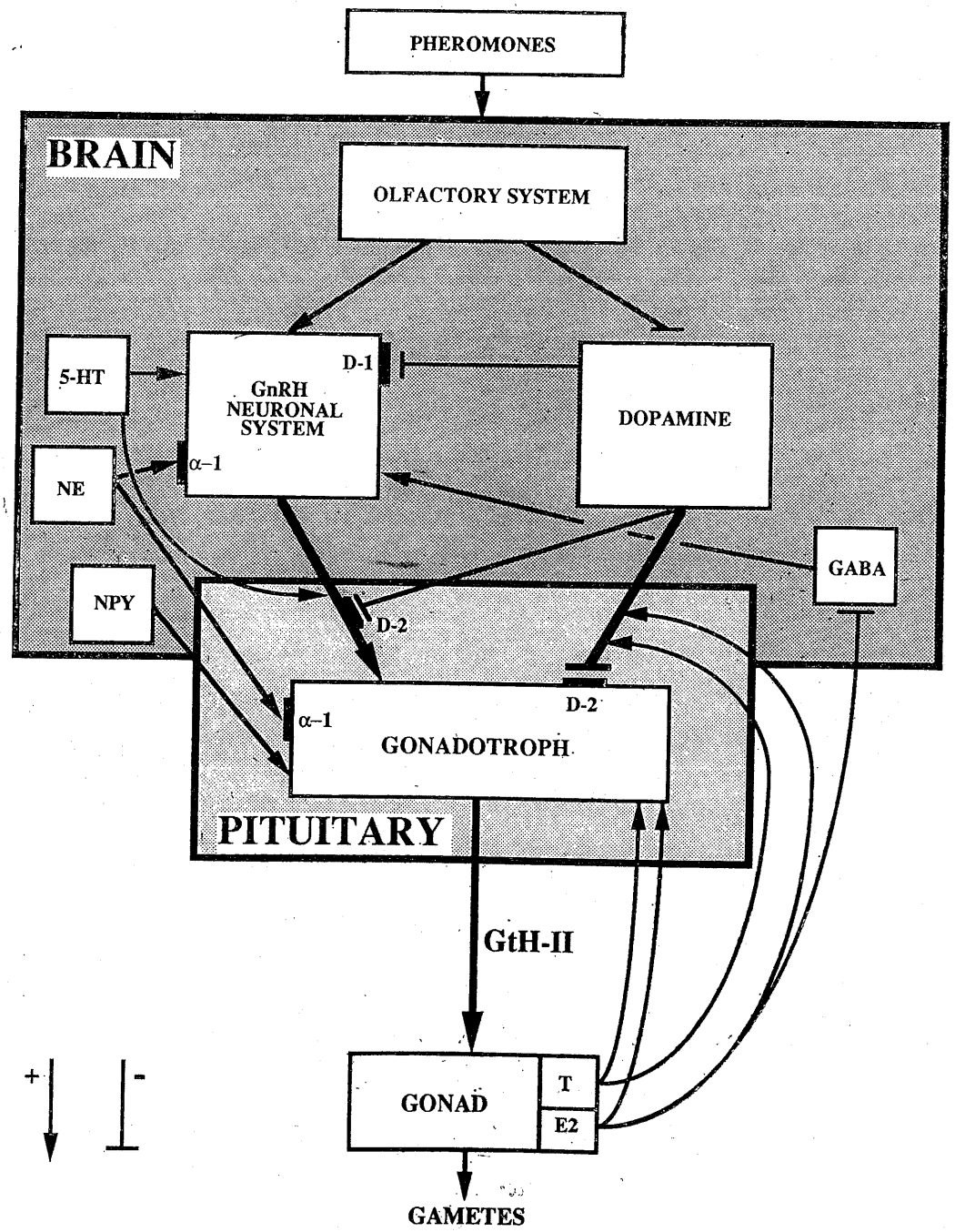

Fig. 1. Proposed model for the neuroendocrine regulation of gonadotropin release in teleosts. A line with an arrow indicates a stimulatory effect; a line with a bar indicates an inhibitory effect. Abbreviations: α_1-noradrenergic receptor, α-1; estradiol, E2; γ-aminobutryic acid, GABA; gonadotropin-II, GtH-II; norepinephrine, NE; neuropeptide Y, NPY; serotonin, 5-HT; testosterone, T; type-1 dopamine receptor, D-1; type-2 dopamine receptor, D-2.

CONCLUSION

The neuroendocrine regulation of reproductive cycles in teleost fish is highly complex. A model of the neuroendocrine regulatory system, largely based on results of studies on the goldfish is presented in Fig. 1. While this model shows the known factors, reviewed above, involved in the regulation of GtH secretion, it does not indicate how the relative importance of these factors may change during a reproductive cycle. Clearly this is a developing model, and it cannot be taken as definitive. However, the model can be used to help understand the interactions of the various neuroendocrine factors influencing GtH secretion in teleosts.

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